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Review

The chromosome peripheral proteins play an active role in chromosome dynamics

Sachihiro Matsunaga and Kiichi Fukui*

Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita 565-0871, Osaka, Japan

*Corresponding author
e-mail: kfukui@bio.eng.osaka-u.ac.jp

Abstract

The chromosome periphery is a chromosomal structure that covers the surface of mitotic chromosomes. The structure and function of the chromosome periphery has been poorly understood since its first description in 1882. It has, however, been proposed to be an insulator or barrier to protect chromosomes from subcellular substances and to act as a carrier of nuclear and nucleolar components to direct their equal distribution to daughter cells because most chromosome peripheral proteins (CPPs) are derived from the nucleolus or nucleus. Until now, more than 30 CPPs were identified in mammalians. Recent immunostaining analyses of CPPs have revealed that the chromosome periphery covers the centromeric region of mitotic chromosomes in addition to telomeres and regions between two sister chromatids. Knockdown analyses of CPPs using RNAi have revealed functions in chromosome dynamics, including cohesion of sister chromatids, kinetochore-microtubule attachments, spindle assembly and chromosome segregation. Because most CPPs are involved in various subcellular events in the nucleolus or nuclear at interphase, a temporal and spatial-specific knockdown method of CPPs in the chromosome periphery will be useful to understand the function of chromosome periphery in cell division.

Keywords: cell division; chromosome dynamics; chromosome periphery; chromosome surface; nucleolar proteins.

Introduction

The chromosome is one of the most dynamic subcellular structures, which is formed at the beginning of cell division when extended chromatin is condensed. Chromosomes then align along the spindle equator and move to the opposite spindle poles. After equal separation, the chromosomes become dispersed and nuclei are reconstructed. Schneider first described chromosomes during mitosis in 1873 (1) and since this time many researchers have been fascinated by the aesthetic dynamics of chromosomes during cell division. Although over 130 years have passed since Schneider's discovery, chromosome dynamics is still a hot topic in biology.

Most organelles have single or double membranes to separate them from the cytoplasmic environment. In contrast to the nucleus, which has the nuclear envelope consisting of a double membrane, chromosomes have no membranes. However, chromosomes do have a distinct surface layer, the chromosome periphery. The chromosome periphery covers chromosomes, including the regions between two sister chromatids. The chromosome periphery is also known as the chromosome surface, chromosome pellicle, perichromosomal matrix, perichromosomal layer, perichromosomal region and perichromosomal sheath. The chromosome periphery is widely found in eukaryotes, including insects, animals and plants (2). The peripheral or related structure of nucleoid in bacteria is not reported (3).

Based on studies of nucleoli in maize, McClintock suggested that the chromosome periphery could play a functional role (4). However, although the chromosome periphery is known as a conserved structure in eukaryotes, its function is not yet known (5–7). Recent knockdown analyses of some chromosome peripheral proteins (CPPs) including histone H1.X, nucleolin, nucleophosmin (NPM), PinX1 and RRS1 have, however, have begun to reveal the secrets of the chromosome periphery. Here, we review the current understanding of the chromosome periphery and CPPs, and propose a new hypothesis that chromosome periphery actively functions in chromosome dynamics.

What is the chromosome periphery?

The chromosome periphery was first described by Strasburger (8). He reported that nucleolar components unite with the chromatin or chromosomes before the breakdown of the nuclear envelope. After this description, cytological analyses revealed the morphological character of the chromosome periphery. The presence of a layer around chromosomes during mitosis was confirmed by light microscopy (9–11). Autoradiography revealed that nucleolar proteins, ribonucleoproteins, were localized in the chromosome periphery (12). Electron microscopy demonstrated that nucleolar proteins coated mitotic chromosomes until nucleolar reconstruction (13–15). Moreover, the chromosome periphery consists of closely packed dense granules which are often in direct contact with chromatin (16). Immunostaining with antibodies against several CPPs and localization analyses of GFP-fused CPPs also demonstrated that the chromosome periphery is not an experimental artifact but a chromosome structure (7, 17). Recent proteome analyses of metaphase chromosomes

revealed that human chromosomes consist of four layers, including the chromosome periphery (17). Proteomic analyses under different conditions indicated that CPPs remain to be associated even with highly purified chromosomes. In contrast, most chromosomal coating proteins, including mitochondrial or endoplasmic reticulum (ER) proteins, are lost from chromosomes under the same conditions (17, 18). This suggests that the chromosome periphery is maintained as a chromosomal structure, resisting isolation and purification procedures or mechanical treatments.

Classification of chromosome peripheral proteins (CPPs)

CPPs in mammalians, listed in Table 1, belong to nuclear or nucleolar proteins at interphase. Most CPPs among more than 30 CPPs are derived from the nucleolus. Most nucleolar-derived CPPs begin to be localized in the chromosome periphery immediately after nucleoli disassembly and are transferred to reassembled nucleoli at telophase. In contrast, a heterogeneous nuclear ribonucleoprotein, hnRNP2A (35), and a large preribosomal complex-associating protein, NO66 (46), are localized in the chromosome periphery only at anaphase. A group of nuclear envelope components is specifically localized in the chromosome periphery at telophase (19). The chromosome periphery at telophase, which is involved in nucleus reassembly, has different characteristics from that at prophase to anaphase. Analyses of barrier-to-autointegration factor (BAF) revealed that the chromosome periphery at early telophase has a distinct core region consisting of a BAF scaffold for the accumulation of nuclear envelope components, including LAP2α, emerin and lamin A (19, 21, 40). Fluorescence recovery after photobleaching (FRAP) analyses of GFP-BAF demonstrated that BAF forms an immobile structure in the core region of the telophase chromosome periphery (21).

The function of most CPPs at interphase is mainly in RNA-related events, including RNA processing, RNA metabolism, ribosome assembly, transcription and splicing. Interestingly, the apoptosis regulator, B-cell lymphoma-2 (Bcl-2) is a member of the CPPs (65, 66). Bcl-2 functions mainly in mitochondria as a negative regulator against cell death progression, a function that is not directly related to RNA-related pathways. Microscopic studies have revealed the interphase localization of Bcl-2 at multiple subcellular localizations: in nuclear outer membrane, nucleoplasm, endoplasmic reticulum membrane and mitochondrial membranes (67, 68). Coimmunostaining with Ki-67 and nucleolin confirmed Bcl-2 colocalization in the chromosome periphery during mitosis but Bcl-2 function on the chromosome periphery remains unknown (22).

CPPs function in chromosome alignment, segregation and spindle assembly

Although the presence of the chromosome periphery has been established, its function has remained elusive. Several roles of the chromosome periphery have been proposed (2, 6, 7). First, it might act as a structural barrier to protect the chromosome from cytoplasmic components. Although the chromosome periphery is not rigid like an exoskeleton, large molecules cannot penetrate into chromosomes. This suggests that the chromosome periphery coats and insulates chromosomes from the surrounding cytoplasmic materials (69). Second, a role in chromosome condensation was proposed based on the temporal and spatial correlation of condensation with the accumulation of some CPPs on chromosomes (70, 71). Moreover, the chromosome periphery might also help maintain the compacted state of chromatin because the chromosome periphery appears to be in contact with chromosomes during mitosis (16). By contrast, recent research has revealed that the chromosome periphery has no relationship with chromosome condensation because condensins have been shown to play the main role in chromosome condensation as internal scaffolds (72). In fact, knockdown of a series of CPPs including histone H1.X, nucleolin, NPM and RRS1 does not influence chromosome condensation (18, 30, 49, 60).

The most credible hypothesis of the function of the chromosome periphery is as a carrier of subcellular components to daughter cells through cell division (2, 13, 16, 73, 74). This hypothesis is supported by the fact that the chromosome periphery contains most of the nucleolar proteins. Nucleoli disassemble at the beginning of mitosis and they begin reassembly at anaphase. Nucleolar proteins in the chromosome periphery are equally transported to the two daughter cells and are incorporated into the nucleoli within the reconstructed nuclei. Based on such dynamic movement of nucleolar proteins on chromosomes, traditional researchers have stated that all forms of nucleoli could be directly derived from the chromosomes (75). McClintock proposed that the release of nucleolar substance from the chromosome periphery might be necessary before the chromatin can again function properly (4).

Chromosome passenger proteins such as Aurora B, borealin, INCENP and survivin are also known to use chromosomes as a vessel in cell division (76). These proteins bind to chromosomes at prophase and detach from metaphase plate-localized chromosomes during metaphase. At anaphase, they form central spindles and at telophase they function in the formation of cleavage furrow. Transportation on chromosomes enables proteins to reach the correct amount and position of future contraction sites. Based on the analogy with passenger proteins, it has been argued that CPPs attached to the chromosome within the chromosome periphery are prevented from dispersal into the cytoplasm. Therefore, CPPs seemed to have no active function on the chromosome; they merely use the chromosome as a means of transport to daughter cells (77). However, the function of the chromosome periphery might not be so simple to the carrying of subcellular components into two new daughter cells. Most nucleolar proteins, after nuclear envelope breakdown, are dispersed in the cytoplasm and are then packed into small organelles, nucleolar-derived foci (NDFs) at anaphase (5, 6). NDFs are equally distributed into the two daughter cells and fuse to become a prenucleolar body at

Table 1 Chromosome peripheral proteins in mammalians.

Protein name	Interphase localization	Duration	Function	References
BAF (BANF1)	Nuclear	T	Nuclear assembly DNA binding protein DNA synthesis progression	(19–21)
Bcl2	Nuclear, mitochondria, endoplasmic reticulum	P → A	Apoptotic regulation	(22)
BCR	Nuclear	P → T	Chronic myelogenous leukemia Serine/threonine kinase GTPase-activating protein	(23)
BOP1	Nucleolar	PM → ET	Ribosome biogenesis	(24, 25)
CRFG (GTBP4, NGB, NOG1)	Nucleolar	PM → LA	GTP binding protein Chronic renal failure	(26)
EBP2	Nucleolar	PM → T	Epstein-Barr nuclear antigen 1-binding protein	(27)
Emerin	Nuclear	T	Component of nuclear membrane	(19, 21)
Fibrillarin	Nucleolar	PM → ET	Processing preribosomal RNA Nuclear morphogenesis Autoimmune disease scleroderma	(28–32)
FLJ23637	Nucleolar	PM → LA	WD repeat-containing protein	(33)
hnRNP A2	Nucleolar	A	Regulation of mRNA metabolism Telomere maintenance	(34, 35)
H1.X	Nucleolar	PM → ET	Mitotic progression Microtubule-kinetochore attachments	(36)
Ki-67	Nucleolar, nuclear	P → T	Cell proliferation Organization of chromatin structure	(37, 38)
Ku70/80 complex	Nuclear	P → T	DNA-PKcs-dependent double-strand break repair Non-homologous DNA end joining Telomere maintenance	(39)
Lamin A (LMNA)	Nuclear	T	Component of nuclear matrix Regulation of nuclear stability Regulation of gene expression	(19, 21)
LAP2α (LAP2A)	Nuclear	T	Nuclear assembly	(21, 40)
MPHOSPH10 (CT90, MPP10P)	Nucleolar	PM → ET	U3 small nucleolar ribonucleoprotein complex rRNA processing	(41)
NAT10 (KIAA1709)	Nucleolar	PM → LA	N-acetyltransferase Regulation of cytokinesis in midbody Acetylation of microtubules	(33, 42)
NIFK	Nucleolar	P → T	Cell proliferation	(43)
No55	Nucleolar	PM → T	Prostate cancer	(44, 45)
NO66	Nucleolar	A	Jumonji family histone demethylase Osteoblast differentiation and bone formation	(46, 47)
Nop52	Nucleolar	PM → ET	Pre-rRNA processing	(24, 32)
Nrap	Nucleolar	PM → ET	Pre-rRNA primary transcription	(48)
Nucleolin	Nucleolar	PM → ET	Pre-rRNA processing Cytoplasmic–nucleolar transport Mitotic progression Microtubule-kinetochore attachment	(31, 49, 50)
Nucleophosmin (NPM, B23)	Nucleolar, nuclear	PM → ET	Ribosomal assembly and transport Mitotic progression Centrosome duplication Microtubule-kinetochore attachment Nuclear morphogenesis	(28–30, 50, 51)
RBBP6 (PSP-R)	Nucleolar	PM → ET	Retinoblastoma binding protein Camptothecin-induced apoptosis mRNA processing Ubiquitin-like pathways	(52, 53)

Table 1 (*Continued*)

Protein name	Interphase localization	Duration	Function	References
Pescadillo (PES1)	Nucleolar, nuclear	PM → ET	DNA replication Ribosome biogenesis Transformation and immortalization	(54, 55)
PinX1	Nucleolar	PM → ET	Mitotic progression Chromosome segregation Microtubule binding protein	(56, 57)
RH-II/Gu	Nucleolar	PM	RNA helicase	(58)
Ribosomal protein S1	Nucleolar	M → A	Component of the small ribosomal subunit	(59)
RRS1	Nucleolar	PM → ET	Mitotic progression Centromeric protection Ribosome biogenesis	(60)
SURF-6	Nucleolar	PM → ET	DNA and RNA binding protein Processing of rRNA	(61)
Tsg118	Nucleolar	PM → ET	Testis development	(62, 63)
U-snRNP	Nucleolar	M → ET	Component of the spliceosome Pre-mRNA splicing	(64)

P, prophase; PM, prometaphase; M, metaphase; A, anaphase; LA, late anaphase; T, telophase; ET, early telophase.

telophase. With regard to the equal delivery and precise recruiting of nucleolar components, NDFs or another delivery system could replace the function of the chromosome periphery. In yeast, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, whose nuclear envelope never breaks down during cell division, there is no chromosome periphery and nucleoli are not dispersed during mitosis (78). Rather, they are partitioned and divided between two daughter cells (5, 6).

Since the application of RNA interference (RNAi) to mammalian cells (79), knockdown analysis with RNAi has been a powerful tool to study the function of essential proteins. This is because knockdown experiments can maintain a minimum level of protein to enable the cell survival, unlike knockout experiments. Depletion of nucleolin, one of major nucleolar and CPPs, was performed by RNAi (27). Nucleolin depletion caused the disappearance of other nucleolar proteins, including fibrillarin and NPM, from the chromosome periphery, suggesting that nucleolin recruits CPPs to the chromosome periphery. This depletion results in mitotic delay, which is then caused by activation of the spindle checkpoint. In fact, nucleolin depletion induced two types of pronounced defect in chromosome congression: misalignment and non-alignment. In the misalignment phenotype, several chromosomes remained near the spindle poles, although the other chromosomes aligned at the spindle equator, whereas, in the non-alignment phenotype, most of the chromosomes remained dispersed. These findings demonstrate that nucleolin is involved in normal chromosome congression. Why does nucleolin absence from the chromosome periphery induce mitotic delay? Detailed analyses of kinetochore-microtubule attachments and dynamic analyses of chromosome oscillation in living cells revealed that the failure of chromosome congression is due to an inability of the kinetochore-microtubule interactions to maintain sufficient tension. This is the first report showing that a CPP can contribute to chromosome congression during mitosis.

This function of nucleolin in the kinetochore seems to be inconsistent with the definition of the chromosome periphery, based on the localization pattern of CPPs. The chromosome periphery was not thought to cover the centromeric region because previous cytological analyses showed that immunolocalization of CPPs could not be detected in centromeric regions (69, 73). Such exclusion from centromeres was thought to be due to the accumulation of kinetochore proteins (2). Only Ki-67 was reported to be localized to centromeric regions of the chromosome periphery (58, 80). However, recent immunostaining results using antibodies against fibrillarin, nucleolin, NPM, PinX1 and RRS1 demonstrated their localization in the centromeric regions of the chromosome periphery, including in the vicinity of the outer kinetochore (Figure 1) (28–30, 49, 57, 60). A new protocol for metaphase chromosome spread preparation (81) contributed to the detection of CPPs including fibrillarin, histone H1.X, NPM, nucleolin and RRS1, around the centromeric region.

Following the discovery of nucleolin function in the chromosome periphery, other CPPs, including histone H1.X, NPM, PinX1 and RRS1, have also been shown to function in chromosome congression (28–30, 57, 60, 57, 82). Knockdown of a variant of histone H1, H1.X, and a centrosome-binding protein, NPM, induces defects in kinetochore-microtubule attachments similar to nucleolin depletion, resulting in a prolonged time from nuclear envelope breakdown to chromosome alignment (28–30, 82). Depletion of a ribosome biogenesis regulatory protein, RRS1, induces the disappearance of Shugosin 1, which is responsible for centromeric protection (60). This results in premature sister chromatid separation. PinX1 was identified by its interaction with the telomere maintenance complex and also interacts with nucleolin in the chromosome periphery and has the ability of microtubule binding (56). Depletion of PinX1 by RNAi induced mitotic delay, which resulted from a defect in kinetochore-microtubule attachment and chromosome segregation (57).

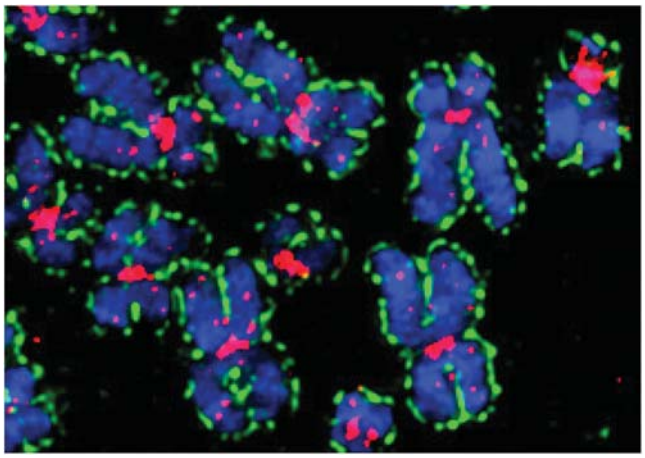

Figure 1 The chromosome periphery in human chromosomes.
The spread of metaphase chromosomes was prepared from synchronized HeLa cells with colcemid using Cytospin 4 Cytocentrifuge (Thermo Scientific, Waltham, MA, USA). The chromosome spread was stained with DAPI in blue and immunostained with antibodies against a chromosome periphery protein, nucleolin in green and a centromeric protein, Aurora B, in red, as described previously (49). The immunofluorescent image was acquired using a deconvolution microscopy system (DeltaVision; Applied Precision, Issaquah, WA, USA). The chromosome periphery covers all the surface of mitotic chromosomes including telomeric and centromeric regions.

Depletion of the chromosome periphery also induced aberrations of mitotic spindles. When nucleolin, H1.X, NPM and RRS1 were knocked down, disorganized spindles and multipolar spindles were observed in the depleted cells (28–30, 49, 60, 82). Without centromeres or kinetochores, only DNA-coated beads could assemble the bipolar mitotic spindle in *Xenopus* egg extracts (83). Taken together, the chromosome periphery might be involved in spindle assembly. Further functional analyses to reveal the interaction and several complexes of CPPs will reveal the structural and functional significance of the CPP network on the chromosome periphery in cell division.

Expert opinion

Most CPPs are multifunctional proteins. For example, some CPPs are involved in transcription and translation. In functional analyses using RNAi, secondary effects, such as a reduction in RNA metabolism and protein synthesis, were ruled out by confirmation that the expression of proteins other than CPPs was not changed using Western blotting with several antibodies (49, 82). However, the side effect of RNAi cannot be excluded completely unless the proteome analysis is performed to confirm all protein expression level. To identify the roles of CPPs in the chromosome periphery, a temporal and spatial-specific knockdown method, for example, chromophore-assisted laser inactivation with a SNAP-tagged protein (84) or Killer-Red protein (85), would be effective.

The chromosome periphery includes some RNAs (86, 87). They are not thought to be transcribed during mitosis but to have other functions in the chromosome periphery. Because a part of CPPs including nucleolin and fibrillarin have an RNA recognition motif (28–30, 49), they could form a complex with RNA in the chromosome periphery but the CPP-RNA network needs to be elucidated by future studies.

Outlook

The future study of the structure and function of chromosome periphery will provide new insight, which could be applicable to the following processes.

1. Regulation of cell division The chromosome periphery has important roles in cell division events, including chromosome alignment, spindle assembly and chromosome segregation, as mentioned above. Temporal or spatial regulation of CPPs will produce more limited effects in chromosome dynamics rather than directly binding proteins to DNA and microtubules. Furthermore, regulation of the chromosome periphery in the size and localization pattern will contribute to the development of artificial chromosomes or mitotic spindles (88–90).

2. Subcellular transport The chromosome periphery can distribute subcellular components equally to daughter cells. Using the chromosome periphery as a transporter will make it possible to exactly distribute substances, including medicines and chemicals to the next generation through cell division. In particular, nucleolar reassembly is dependent on components in the chromosome periphery. The chromosome periphery consists of only certain nucleolar proteins. Regulation of the amount of specific nucleolar proteins in the chromosome periphery would contribute to nucleolar modification in the component balance, leading to indirect regulation of RNA transcription and protein synthesis (5, 6, 91).

Highlights

The chromosome periphery covers the entire surface of chromosomes, including centromeric and telomeric regions. The chromosome periphery has at least three functions, as an insulator or barrier to protect dividing chromosomes from subcellular substances, as a carrier of nuclear and nucleolar

components for equal distribution to daughter cells and as a regulator of mitotic progression, involving cohesion of sister chromatids, kinetochore–microtubule attachments, spindle assembly and chromosome segregation. The detailed structure and function of the chromosome periphery will be elucidated by analyses of CPPs using temporally and spatially controlled experiments.

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